Remarks

Please amend claims 1, 5, 6, 12-14, 16-18 and 31, and cancel claims 2-4 and 15 without prejudice or disclaimer to the subject matter therein. Following this amendment claims 1, 5-6, 9-14, 16-18, 29-31, 35-36, 39 and 42 will be pending. Support for the claims as amended may be found for instance, on page 13, lines 1-2, on page 15, lines 6-8, claims 3 and 4 as originally filed, the examples and throughout the application as originally filed. No new matter is added as a result of this amendment. Applicant believes the current amended claims and remarks below address the Examiner's remaining concerns.

Summary of the Office Action

The Office Action rejected claims 1-6, 9-18 29-31, 35-36 and 42 as being unpatentable under 35 U.S.C. § 103(a) over Laustsen et al. (U.S. Patent 6,080,564) in view of Larsen et al. (WO 95/29999), Heinsohn et al. (U.S. Patent 5,215,908), Ward et al. (*Biotechnol* 8:435-440, (1990) and Thomas et al. (J Agric Food Chem 32:825-828). Further, claim 39 was further rejected in view of Kappeler et al. (2002/0164696 A1).

Objections

Claims 12 and 15 were objected to for misspellings or improper dependency. Applicant appreciates the Examiner's suggestions and have amended the claims in accordance with those suggestions.

35 U.S.C. §103(a)

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The claimed invention is directed to a method of providing a milk clotting composition comprising providing a medium having a pH of 2.0 or higher that comprises chymosin and glucoamylase and subjecting said medium to a pH between about 1.8 and about 2.0 for a period of time of at least 2.5 hours to inactivate at least 50% of enzymatic activity of said glucoamylase

while maintaining at least 85% of the enzymatic activity of said chymosin. Claim 1 now recites a time period of "at least 2.5 hours to inactivate at least 50% of said glucoamylase while maintaining at least 85% of the enzymatic activity of said chymosin".

Applicant submits that the claimed characteristics of the methods render patentable the claimed invention. Additionally, Applicant provides further arguments, which distinguish the claimed invention from the prior art of record.

The Examiner continues to reject the claims over the references of record, further stating on page 8 of the Office Action that:

> [i]rrespective of the purposes of the individual prior art references, they nonetheless combine to provide motivation to practice the claimed invention. Contrary to applicant's assertion, there is clear motivation to combine the cited references, which is to reduce contaminating enzyme activities, e.g. glucoamylase activity, activate the expressed chymosin, and to stop fermentation and cell growth of the cultured cells, all in a single step as described above. This motivation is specific to the limitations of chymosin as a protein that maintains partial activity and glucoamylase as the undesired protein.

It was, in essence, asserted in the Office Action that the purpose of each of the cited references does not matter so long as they "combine" to "provide motivation to practice the claimed invention". (Office Action, page 8). This assertion appears to be based on impermissible hindsight to construct the claimed invention from portions of prior art selected without a suggestion in the prior art to do so. This assertion is erroneous as a matter of law. The motivation to combine the references must come from the references themselves not from reading Applicant's specification or claims. The teaching or suggestion to combine and the reasonable expectation of success must both be found in the prior art, and not in Applicant's disclosure. M.P.E.P. § 2142. In contrast, the assertion in the Office Action is based on Applicant's own specification which for the first time presents all Applicant's claimed elements to obtain the claimed method of making the milk clotting composition.

Laustsen was cited as teaching individually several aspects of the claimed invention, but it was admitted that "Laustsen et al. do not specifically teach chymosin as a desired polypeptide, glucoamylase as an undesired polypeptide, and the use of a pH of less than 2.0 to reduce glucoamylase enzyme activity." (Office Action, page 5). The Examiner cites Larsen as teaching a "treatment of crude extract comprising chymosin with an organic or inorganic acid at a pH of as low as (0.5)..., which results in activation of the chymosin." Id. According to the Office

Action, the cited references are directed toward a variety of characteristics related to enzymes, enzymatic activities or uses, for instance, separating milk clotting enzymes (Larsen), purifying and recovering chymosin (Heinsohn), inactivating labile proteases in media with Aspergillus species (Laustsen), use of chymosin expressing hosts (Ward), and recognizing that glucoamylase is a contaminant in chymosin (Thomas).

Applicant now discusses each of the references alone and in combination, although Applicant submits that any combination of the references is improper. Initially, the Office Action on page 4 states "Laustsen et al. teach a method of obtaining a desirable enzyme with inactivated undesired enzyme activities by treatment with low pH (Column 1, top)"; however, Laustsen actually discloses "[t]he present invention relates to a method for inactivation of one or more undesirable enzymes in a mixture of enzymes containing a desirable enzyme." (Column 1, lines 11-13). Therefore, Applicant respectfully points out that that portion of Laustsen does not include a statement related to using any pH, much less a low pH. Laustsen's disclosure, as a whole, is directed to:

a method of inactivating at least one undesirable enzyme in a mixture of enzymes containing a desirable enzyme comprising:

a) holding the mixture at 2°C to 75°C at pH below 5.0 for at least 20 sec.; and/or

b) holding the mixture at 2°C to 75°C at pH above 9.0 for at least 20 sec. Col. 1, ll. 48-55.

Further, the Office Action cites the following as teachings in Laustsen:

> Laustsen et al. teach a method of determining optimal conditions (pH, time, and temperature) for obtaining a desired polypeptide with inactivated undesired enzymatic activities by determining the pH optimum of the desired polypeptide (column 4, top). Laustsen et al. teach it is advantageous to hold the pH as acidic as possible for a desired polypeptide with an acidic pH optimum (column 4, top).

Again the reference actually discloses a pH range of "below 3.5" to "above 10.0". Col. 4, lines 17-20. Further, the Office Action states Laustsen does not "specifically teach chymosin as a desired polypeptide, glucoamylase as an undesired polypeptide, and the use of a pH of less than 2.0 to reduce glucoamylase enzyme activity." (Office Action, page 5). Therefore, this reference is actually deficient in many aspects as related to the claimed invention in that it fails to disclose or suggest: chymosin, glucoamylase, a pH range of about 1.8 to about 2.0 for treating the

medium, a time period of at least 2.5 hours to inactive glucoamylase, inactivating at least 50% of glucoamylase activity, and maintaining at least 85% of the chymosin activity. This said, it is difficult to appreciate where the motivation in this reference, without the benefit of Applicant's disclosure, exists to be combined with the other references cited to achieve the Applicant's claimed invention.

The U.S.P.T.O. cites Larsen as teaching that chymosin maintains "enzymatic activity at a pH at or below 2.0" as a fact well known in the art. (Office Action, page 5). Larsen et al. is directed to

> "[a] process of separating milk clotting enzymes in extracts of animal stomach tissue which comprises contacting a partially purified extract with an ion exchange resin under conditions where chymosin is bound to the resin and recovering the chymosin, the process optionally comprising the further step of recovering pepsin, from a pepsin-rich extract fraction using an ion exchange resin, and a liquid of powdered rennet composition containing at least 90% of its milk clotting activity as chymosin activity, the composition comprising a chymosin stabilizing agent which is typically selected from a protein, a peptide, an amino acid or ascorbic acid." (See, Abstract).

Applicant submits that there may be several ways to separate chymosin from undesired enzymes, and Larsen is directed to one of those ways. Larsen does indicate that chymosin may be active at pH's below 2. Indeed, Larsen suggests pH's as low as 0.5 and as high as 5.0 to convert pre-enzymes (such as prochymosin or pepsin pre-enzyme) to endopeptidases (such as chymosin or pepsin), p. 10, lines 21-22. This disclosure fails to suggest the Applicant's claimed invention at least because the claimed pH range is between about 1.8 and about 2.0 and because the pH used in the claimed invention is not for "activating" pre-enzymes, rather it is for at least partially inactivating a particular, undesirable enzyme, glucoamylase. Larsen therefore alone or in combination with Laustsen fails to provide motivation to achieve the claimed invention. Indeed, one would not be motivated to combine a reference that fails to disclose chymosin, glucoamylase, the specified pH range, or time period for treating the chymosin-containing medium (Laustsen) with a reference (Larsen) that fails to disclose glucoamylase, the claimed pH range, the claimed time period and is directed to separating chymosin from pepsin using pH extraction methods (See, Larsen, Summary of the Invention).

The U.S.P.T.O. cites Heinsohn as teaching "treating an *Aspergillus* culture growth medium comprising chymosin with a pH of about 2 in order to stop fermentation and cell growth

of the cultured cells as a first step in the purification of the chymosin (column 2, bottom)." (Office Action, page 5). Heinsohn is directed to "the recovery and purification of chymosin from aqueous mixtures of enzymes, particularly aqueous mixtures of enzymes produced by fermentation processes" (Col. 1, lines 9-12). Further the reference discloses "...a method for separating chymosin from an aqueous mixture of enzymes by contacting the aqueous mixture with a phenyl-sepharose resin to bind the chymosin to the resin and separating the resin and the bound chymosin from the remainder of the aqueous mixture." (Col. 1, lines 51-56). Heinsohn fails to disclose glucoamylase, the recited pH range or the treatment time period of the Applicant's claims. Therefore, this reference alone or in combination with Larsen and Laustsen fails to suggest the Applicant's claimed invention, and certainly does not provide motivation to combine it with Laustsen and Larsen for any purpose, much less to obtain the claimed invention. Indeed, Heinsohn's "essential feature of the process however, is the use of phenyl-sepharose resin" (Col. 3, lines 36-37) which allows Heinsohn to separate out chymosin as "[i]t has been found quite surprising in the present invention that chymosin is essentially the only enzyme material present in a fermentation broth that will bind to the sepharose resin under low pH and/or high salt concentration conditions." (Col. 3, lines 55-59). Given these statements Applicant fails to understand where the motivation to combine Heinsohn with Laustsen and/or Larsen exists in this reference or in Laustsen and/or Larsen to arrive at the Applicant's claimed invention. Any improper combination of the three references would have included the phenyl-sepharose resin which would have selectively bound the chymosin. However, such a combination would not have included:

> the treatment of the medium at pH of about 1.8 to about 2.0 for a time period of at least 2.5 hours;
> inactivation of at least 50% of the glucoamylase enzymatic activity; and
> retention of at least 85% of the chymosin's enzymatic activity.

Even if, *arguendo*, glucoamylase existed in the fermentation broth, one would not have to inactivate it as the phenyl-sepharose resin would separate out the chymosin and eliminate the need for further purification.

The U.S.P.T.O. cites Ward for the proposition that "recombinant expression of chymosin using various hosts was well known in the art at the time of the invention." (Office Action, page 5). However, Ward again does not disclose or suggest a method of making a milk clotting composition by reducing the pH of a medium containing chymosin and glucoamylase to the

claimed range of about 1.8 to about 2.0, or the resulting glucoamylase or chymosin activity. Indeed, Ward is directed to recombinants of chymosin and does not appear to discuss how to separate out the glucoamylase from the chymosin. Indeed, on page 439, top Ward states:

> Loss of the fusion protein and an increase in active chymosin concentration could be induced simply by lowering the pH of samples to 2. As would be expected if processing was dependent on chymosin activity, at least some of the chymosin released from the fusion protein under these conditions appeared to be in the form of pseudochymosin. Presumably, this would eventually be further processed to mature chymosin under the appropriate conditions. Processing of the fusion protein at pH 2 was inhibited by pepstatin, an inhibitor of chymosin and other aspartyl proteases.

Ward again fails to disclose or suggest the recited pH range, the time period for treatment, or the activity levels following treatment for both chymosin and glucoamylase. The improper combination of Ward with Larsen, Laustsen and Heinsohn would fail to suggest the claimed invention as, per the discussion above each of those references alone or in combination, fails to disclose or suggest the claimed invention, their combination with Ward would also fail to suggest it. Further, Ward fails to provide motivation to be combined with any one or combination of references as it is directed to fusion proteins, not the inactivation of at least 50% of glucoamylase in a composition comprising chymosin, while preserving at least 85% of chymosin activity.

Finally, the USPTO cites Thomas for the proposition that the "presence of glucoamylase enzymatic activity as a contaminant in preparations of chymosin was well known in the art at the time of the invention." (Office Action, page 6). Applicant's review of the article failed to find reference to glucoamylase, only amylase. Additionally, this reference fails to provide guidance or the motivation for one skilled in the art to achieve the remaining elements of the claimed invention, alone or in combination with the other cited references. For example, the reference fails to disclose or suggest the recited pH range, the time period, or the activity levels of chymosin, much less of glucoamylase. Therefore, Thomas in any improper combination with the other references fails to suggest the claimed invention.

As discussed above, the cited references themselves do not provide the motivation for the combination thereof. Rather, the motivation to combine the references appears to be improperly taken from Applicant's disclosure. Further, as discussed above, even if *arguendo,* the references could be properly combined, they would be no more than an invitation to experiment rather than

providing a reasonable expectation of success as the references fail to disclose or suggest all the claimed elements of the invention as they must do for a proper obviousness rejection. This is particularly true given the recited pH range, the minimum time period to achieve the claimed activity levels and the disparate disclosures cited to reject Applicant's claims. This is underscored by the fact that the references fail to suggest which aspects of each reference should or could be selected to obtain the claimed invention.

Additionally, Applicant respectfully submits that "a combination may be patentable whether it be composed of elements all new, partly new or all old." *Rosemont, Inc. v. Beckman Instruments, Inc.*, 221 U.S.P.Q. 1, 7 (Fed. Cir. 1984). There must be something in the prior art as a whole to suggest the desirability, and thus the obviousness, of making the combination. *Lindemann v. Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 221 U.S.P.Q. 481, 488 (Fed. Cir. 1984). *Interconnect Planning Corporation v. Feil, et al.*, 227 U.S.P.Q. 543, 551 (Fed. Cir. 1985). In the present case there is no such motivation present in the references, rather the motivation is derived from Applicant's specification and claims. The motivation is even further lacking given Applicant's newly recited limitations. Again, the statutory standard of 35 U.S.C. §103 is whether the invention, considered as a whole, would have been obvious to one of ordinary skill in the art, *not* whether it would have been obvious for one of ordinary skill in the art to try various combinations. *Akzo N.V. v. E.I. duPont de Nemours*, 1 U.S.P.Q.2d 1705, 1707 (Fed. Cir. 1987). [Emphasis Added].

Additionally, claim 39, ultimately dependent from claim 1, was rejected further in view of Kappeler which discloses "*Camelus dromedarius* chymosin." (Office Action, page 7). It is respectfully submitted that as claim 1 is non-obvious in view of the five primary references and Kappeler does not provide the motivation to combine the five references or to combine his own reference with the five references, the combination(s) are improper. Further, Kappeler does not supply the Applicant's claim elements missing in the disclosures of the five references. Therefore claim 39 is non-obvious in view of Kappeler.

As claim 1 is non-obvious in view of the references for all the reasons discussed above, each of the claims which depends therefrom is also non-obvious; therefore, all of Applicant's claims are patentable in view of the references of record. The obviousness rejections are respectfully traversed.

CONCLUSION

Applicant asserts that the application is in condition for allowance. Reconsideration and allowance of all pending claims is respectfully requested. Should any outstanding issues remain, the Examiner is invited to telephone the undersigned.

Respectfully submitted,

HUNTON & WILLIAMS

Dated: Aug 9 2004

By: _____

Jeffrey T. Perez
Registration No. 52,110
Stanislaus Aksman
Registration No. 28,562

HUNTON & WILLIAMS
1900 K Street, NW, Suite 1200
Washington, DC 20006-1109
(202) 955-1500 (Telephone)
(202) 778-2201 (Facsimile)